CONTRIBUTIONS TO THE MORPHOLOGY AND LIFE HISTORY OF *PIROPLASMA CANIS*

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Piroplasma canis was discovered by Piana and Galli-Valerio in the year 1895,[20] and, on account of its wide distribution and the ease with which experimental infection can be transmitted from dog to dog, this parasite has been the subject of extensive study. It seems unnecessary to give a complete review of the literature, especially since Nuttall and Graham-Smith,[17-19] and more recently Christophers,[3] have given fairly complete bibliographies.

Additional interest has been attached to Piroplasmata in general since the appearance of Schaudinn's[21] work, in which he mentions (p. 428) Kossel's and Weber's observation, and suggests that *Piroplasma* may pass through a life cycle similar to that of *Halteridium*. However, very little evidence in support of this hypothesis has been brought forward by later workers.

The parasite, on which the following observations were based, was obtained from Professor Uhlenhuth, of Berlin, to whom we have pleasure in expressing our indebtedness. The strain has been kept going in pups and dogs by means of simple inoculation. In our hands it has shown itself very virulent, even in the case of full-grown dogs, and no animal survived the infection. Young dogs showed parasites, in scanty number, two to three days after an intra-peritoneal injection of 1 to 2 c.c. of heavily-infected blood; their number increased very slowly during the following 24 hours; after this a rapid increase in the number of parasites set in. Within 40 hours after the first

appearance of the parasites, the peripheral and especially the blood of the organs was usually teeming with them, and the animal succumbed to the infection. The course of the disease in full-grown dogs was somewhat modified, as the parasites only appeared after a prolonged incubation period, and never in such large numbers as in young animals.

The clinical feature of this disease has been dealt with in full by previous workers. It is a noteworthy fact that in nearly all our cases haemoglobinuria was more or less pronounced, the urine being frequently of a dark port wine colour. Only in very few cases was jaundice well marked.

Technique. All our observations were made on wet films using Breinl's methods. The blood-smears were fixed in strong Flemming's solution, and afterwards stained with safranine and methylene blue, according to Breinl's method, or his modification of Heidenhain,[15] using as counter-stain a dilute solution of Bordeaux red. By this means the cytological details of the parasites were well preserved, an attainment which is impossible by any dry film method.

EARLY FORMS OF PARASITES IN THE BLOOD

The early forms in the blood are usually very large and irregular, frequently exhibiting pseudopodia of varying form and size (figs. 1-5). Some of these processes are so fine that they simulate flagella, and at times small particles of protoplasm appear to become detached; but in most instances a very fine band may still be detected connecting these little masses with the parasite. The protoplasm consists of a fairly coarse spongioplasm ('Schaumplasma'), containing fine, bluish staining granules, embedded in its substance. At this stage the parasites usually possess a single nucleus, in the form of a small, dense, darkly staining mass of chromatin, which is sometimes surrounded by a vacuole, filled with lightly staining substance. The division of these forms is by simple fission. The nucleus of the mother cell elongates, and afterwards separates into two halves which move further apart; meanwhile the parasite itself increases in size, and eventually divides into two daughter cells (figs. 2-4). This process goes on very rapidly.

In the early forms very rarely a second smaller chromatic mass is

present (fig. 5), which may be connected with the main nucleus by a fine darkly staining line. If Breinl's stain is used this smaller nucleus usually takes a dark purplish-blue colour, whereas the nucleus stains dark red. This difference is noticeable, however, only in well stained specimens.

The division of the later forms proceeds in a different way. The small nucleus together with the chromatic line usually divides first. A median cleft afterwards appears, which extends between the two small nuclei. In the meanwhile the large nucleus elongates, and finally separates into two equal halves. The two daughter cells then become separate (figs. 6-9). This division results in the formation of two pear-shaped parasites.

At this stage of the infection, round parasites now and again reproduce by budding, a process which becomes more frequent as the infection advances. The nucleus throws out a portion of its chromatin, which moves outwards, but remains connected with it by means of a thick band. As this chromatic mass approaches the periphery of the parasite, the cytoplasm bulges out from the surface and concentrates itself around the terminal enlargement of the chromatic band. The latter structure becomes thinner and finally breaks. The connection of the bud with the main mass becomes in the meanwhile less and less extensive, and finally the bud is detached (figs. 10-12). Very often two buds are formed at the same time in a similar manner (figs. 13-16). A large number of buds as described by Kinoshita[7] have never been distinctly observed by us.

Schaudinn[21] and Lühe[13] were the first to draw attention to the presence of a small nucleus in *Piroplasma*. This discovery has been confirmed by different workers. Schaudinn named the second nucleus a blepharoplast, and most of the later workers adhere to this view, without, however, producing any evidence in support of it.

Our observations show that but few binucleate parasites are present at an early stage of the infection. This small nucleus then arises from the large one, usually at a later stage of the disease. Different phases of this process may sometimes be seen in one red corpuscle containing several parasites (figs. 17, 18, 42). The nucleus, which at this stage is surrounded by a vacuole, buds off a small part of its substance, which moves to the edge of the vacuole, often leaving a thin connecting line behind.

LATER FORMS OF PARASITES IN THE BLOOD

As the infection advances, the parasites undergo marked changes, and only now and again large amoeboid forms are seen. The parasites diminish in size, and are frequently pear-shaped. The protoplasm, which at first is a typical 'Schaumplasma,' becomes much denser in structure. The percentage of binucleate forms increases, and many free forms are encountered.

A peculiar feature of this stage is the detachment of small parts of the cytoplasm in a definite way. At one side of the cell appears a vacuole, which increases in size and enlarges within the parasite, until the protoplasm is almost separated into two unequal parts, which finally become separate. The smaller part is entirely cytoplasmic in nature (figs. 22-26).

Owing to the rapidity with which multiplication takes place, the nuclear details become very irregular, and frequently a second division commences before the completion of the first (figs. 20, 51-52).

The nucleus of the round forms is usually surrounded by a vacuole (fig. 33). The division is by simple fission, in which the nucleus divides with the vacuole (figs. 33-38).

Sometimes the parasites assume a signet-ring form, a large vacuole occupying the middle of the cell, the nucleus which lies at the periphery often dividing (fig. 21).

The usual mode of division at this stage results in the formation of two pear-shaped forms, but differs from that in the early stages of the disease. Starting again from the round binucleate form, either the large or the small nucleus divides, together with the line; the small nucleus moves to the edge of the parasite, and the chromatic line becomes fainter, and in many instances finally disappears (figs. 45-52). The divided large nuclei frequently remain connected (fig. 46). At this stage one or two vacuoles appear about the middle of the cell, and increase in size. Often the two parasites are connected by three fine protoplasmic strands, two peripheral and one across the middle, a large and small nucleus in each half. First the central connecting strand breaks, and the separation of the two parasites becomes more pronounced, until they are only connected by one strand at their apices. Whilst the pear-shaped forms are still connected in the above described manner, the connecting cytoplasmic

strand may be seen considerably thickened at the middle (fig. 19). This connection becomes smaller and smaller until both parasites separate into two pear-shaped forms; the small nucleus dividing again even before the separation is complete. On the other hand division of the large nucleus may set in first, accompanied or not, by a division of the connecting line (figs. 44-52).

While the pear-shaped forms are still connected, a second small nucleus may arise from the large one (fig. 53 n_2). We have not been able to explain the meaning of this process.

A striking feature of the present stage of the infection is the occurrence of unequal divisions of the parasite. The nuclei of the cells divide in the *usual* way, but the cytoplasm divides into two unequal parts, the smaller parasite assuming a crescent shape. This division may be compared with the sickle-shaped detachment of the cytoplasm described above.

Leishman and Statham[11] describe a similar process in *Leishmania donovani* (*Piroplasma donovani*), with the important difference, however, that eventually nuclei were seen in these detached parts of the parasite.

We, however, could not follow an analogous procedure in *Piroplasma canis* To our minds there are two distinct processes. Either the cytoplasm becomes detached in a regular way without co-operation of the nuclei; or, the nuclei take part in the division. The enucleated particles of cytoplasm probably degenerate, and give rise to the appearance of irregular dark staining masses in the protoplasm of the infected red cells, or the detached part contains one or two nuclei and gives rise to a new parasite (figs. 28 and 29). Rarely chromatin appears to be given off from the nucleus, and become free in the red cell (fig. 30). This process has already been described by Nuttall and Graham-Smith,[18] but its significance is unknown.

The parasites occurring in the blood of organs do not differ markedly from those found in the peripheral and heart blood. As division appears to proceed more rapidly in the organ blood, the parasites are usually slightly smaller and more compact. The free forms, which occur in greater numbers in the organs, divide in the same way as the intra-cellular forms, i.e., round and pear-shaped division. (Compare fig. 43.)

FLAGELLATED FORMS

Flagella-like processes in different species of *Piroplasma* in blood have been frequently described. Bowhill and Le Doux,[2] Nuttall and Graham-Smith,[18] and Kinoshita,[7] describe their occurrence in *Piroplasma canis*; Lignières[12] and Bowhill[1] in cattle piroplasmosis; Fantham[5] in *Piroplasma muris*. These processes have been more frequently observed in cultivation forms, and in developmental forms in the tick, by Koch,[9] Kleine,[8] Kinoshita,[7] and Miyajima.[14]

The meaning of some of these forms has been explained in different ways. Doflein,[4] Nuttall and Graham-Smith,[17] and Hartmann[6] discuss the probability of their being mikrogametes analogous to the mikrogametes of the life cycle in malaria, but nothing in the nature of a proof of this conception has hitherto been brought forward. When we consider the active amoeboid movement of the young parasites, it would certainly appear that most of the flagella-like processes seen must be regarded simply as fine pseudopodia. Kinoshita,[7] on the other hand (figs. 41 and 46), figures a flagellum which arises from a blepharoplast and takes a chromatic stain in the same way as do trypanosome flagella.

Now and again, long flagella-like processes, which were evidently pseudopodia, have been seen in intra-corpuscular forms (figs. 31, 32). (Compare Kinoshita, fig. 9.)

Very rarely true small flagellate forms were seen, especially in blood from the lung; but we were never able to trace the origin of the single flagellum (fig. 27).

Large flagellated forms have been described by Miyajima[14] in cultures of *Piroplasma parvum*, and these forms he describes as intermediate stages in the development of trypanosomes from a typical *Piroplasma*. He discusses at length the possibility of a mixed infection of piroplasmosis and trypanosomiasis of the blood used for his culture experiments, but the facts he brings forward seem to be very much against such a possibility.

Kossel's and Weber's observation, as quoted by Schaudinn,[21] seems to have anticipated Miyajima's observation with regard to large flagellated forms, with the difference that they observed his culture forms in freshly drawn blood.

Nuttall and Graham-Smith[17] in 1905 were the first to describe a

few large forms of *Piroplasma canis*, which simulate the crescents of aestivo-autumnal malaria, having the chromatin sometimes concentrated in the middle, sometimes forming a loose mesh work. These parasites were 10·4 to 10·7 μ long and 1·4 to 1·7 μ broad. They regarded them at first as gametes, but in their last paper they do not consider them to have any connection with *Piroplasma canis*. Their chief reason was the fact that they only found seven 'gametoid bodies' altogether, and these occurred in one animal. No flagella could be observed.

Kinoshita figures somewhat similar parasites (figs. 47, 48, 49) seen in the heart-blood, pancreas and lung, some hours after death. He refers to his figs. 47 and 48 as conjugation forms, and to fig. 49 as an ookinet (?) in accordance with Schaudinn's ideas.

We have been able to trace the development of large biflagellate forms from the normal intra-cellular parasite. In the films, where large biflagellate forms occurred, along with ordinary intra-cellular parasites, forms were also found in which both nuclei were considerably enlarged, as represented in figs. 54, 55. These bodies increase in size, and the smaller nucleus in the meanwhile divides (fig. 56), often remaining connected with the large one by fine chromatic lines. The subsequent changes vary in details, but on the whole two main forms of development may be followed. On the one hand, an irregular number of small round chromatin masses originate from the large nucleus, frequently remaining connected with it by fine chromatic lines, which eventually disappear (figs. 72, 73). From the fact that these masses often appear double, it seems possible that they may divide (figs. 75, 76). At the same time the appearance of the large nucleus changes; the chromatin becomes aggregated at the centre, and a lightly staining area is left between it and the well-defined nuclear membrane (figs. 60, 72-74). Eventually, two flagella are formed, each of which may end in the neighbourhood of a small chromatic mass, but in some cases the flagella appear to have no definite origin (figs. 74, 77, 78).

A second mode of development takes a somewhat different line. The large nucleus frequently buds off at first a small number of granules, and eventually it seems to throw out the whole of its chromatin in form of a large densely staining mass; figures 62-66 representing different stages of this process. The remainder of the

original nucleus persists as a homogeneous lightly staining mass, which retains its original form and moves to one side of the parasite. At this stage usually one long flagellum arises in the neighbourhood of a small chromatic body very often situated at one end of the parasite (figs. 61-64). Shortly after, a second flagellum is formed, sometimes arising in close vicinity to the origin of the first flagellum, sometimes at some distance (fig. 65).

The above described development is very liable to modifications. Occasionally two large masses of chromatin are thrown out of the nucleus (fig. 68), and at the same time these latter sometimes divide (fig. 71).

Whilst these nuclear changes are taking place, the parasites increase in size, and become elongated. The protoplasm changes its appearance, and becomes very loose and vacuolated. The dimensions of fully developed flagellate forms vary between 6 to 8 μ in length and 2 to 3 μ in width.

These forms have been repeatedly observed by us in very small numbers in the peripheral blood of dogs on the day before death. Only in one animal were they abundant, and only in this case have we been able to follow their development. The blood was taken in the morning of the day before death. Films made actually on the day of death did not show any of these forms, neither in the peripheral nor in the heart blood, only two of these flagellated cells being found in organ films (spleen and bone-marrow).

This observation seems to point to the fact that the biflagellate forms of *Piroplasma canis* represent a very transient stage in its life-history. For this reason, it might have been very easily overlooked. We, however, at present, are not able to form a definite opinion as to the significance of this stage in the life-history of the parasite, especially as the subsequent development of the flagellate forms could not be traced.

Up to the present, no observations, either in culture or in the intermediate host, throw any light upon their meaning. Developmental stages of these flagellate forms in some respects resemble those occurring in the development of the flagellate forms in the cultures of *Leishmania donovani*.

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EXPLANATION OF THE PLATES

All the figures are drawn with a Zeiss apochromatic 2 mm. oil immersion lens, aperture 1·40. Oc. 18.

Plate VI

Figs. 1-16.—Breinl's stain.

Fig. 1.—Early amoeboid form.

Figs. 2-4.—Division stages of amoeboid forms.

Fig. 5.—Binucleate amoeboid form.

Figs. 6-9.—Pear-shaped division of binucleate form.

Figs. 10-12.—Stages in the formation of a single bud.

Figs. 13-16.—Stages in the formation of two buds.

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PLATE VII

Figs. 17-27.—Breinl's stain.

Figs. 28-32.—Heidenhain-Breinl's stain.

Figs. 17, 18.—Different stages of the formation of the small nucleus.

Fig. 19.—Pear-shaped division form, showing thickening of the connecting line.

Fig. 20.—Pear-shaped division; division of small nuclei before separation.

Fig. 21.—Signet ring form, with divided nucleus.

Figs. 22-26.—Formation of the sickle-shaped mass of cytoplasm.

Fig. 22.—Appearance of vacuole at the edge of the cell.

Figs. 23, 24.—Growth of the vacuole.

Figs. 25, 26—Separation of the sickle-shaped cytoplasmic part.

Fig. 27.—(*a*) Free pear-shaped binuclear form.
(*b*) Free small flagellate parasite.

Figs. 28, 29.—Unequal division.

Fig. 30.—Extrusion of chromatin into the red cell.

Fig. 31.—Amoeboid form with long pseudopodium.

Fig. 32.—Division of same.

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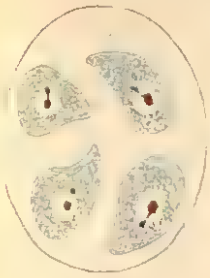

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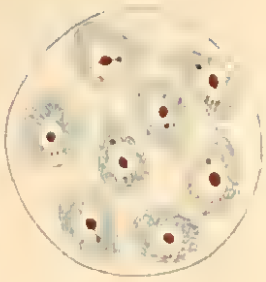

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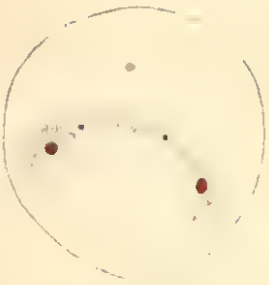

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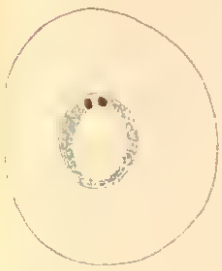

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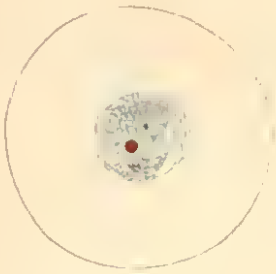

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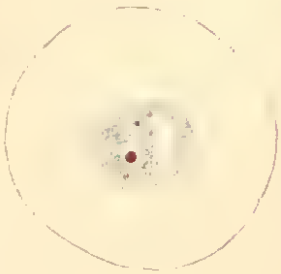

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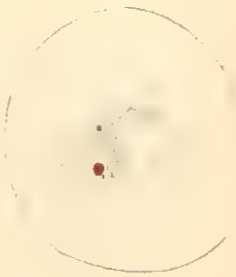

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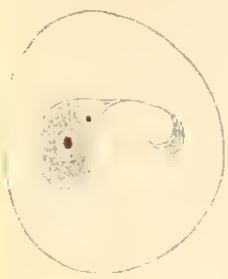

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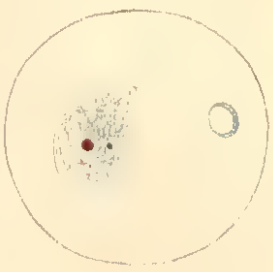

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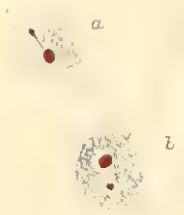

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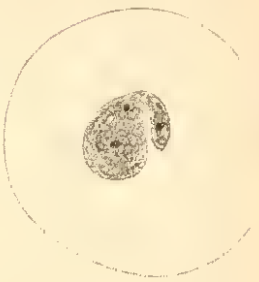

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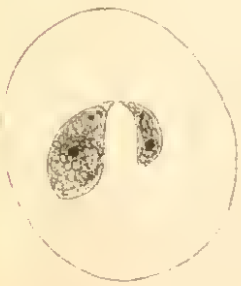

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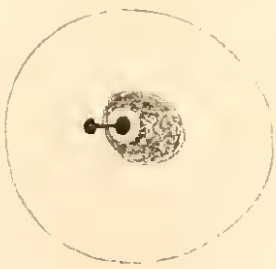

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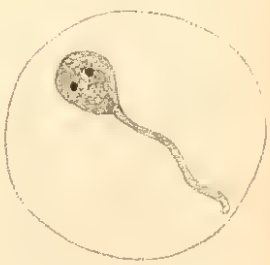

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Plate VIII

Figs. 35-48.—Heidenhain's stain. Later stages of division.

Figs. 33-40.—Division of round forms.

Fig. 41.—Round intra-cellular binucleate form.

Fig. 42.—Formation of small nucleus.

Fig. 43.—(*a-c*) Division of free round forms. (*d*) Formation of small nucleus in free form.

Figs. 44-48.—Stages in the late pear-shaped division.

Fig. 44.—Division of large nucleus and chromatic line

Fig. 45.—In the left parasite division of small nucleus. In right parasite appearance of vacuole and commencement of division of large nucleus.

Fig. 46.—Both nuclei divided; daughter cells connected by three strands.

Fig. 47.—Disappearance of middle connecting strand.

Fig. 48.—Rupture of lower connecting strand.

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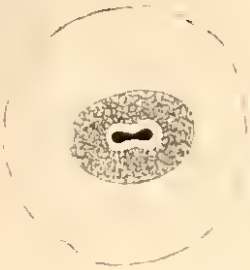

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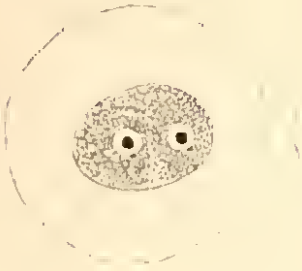

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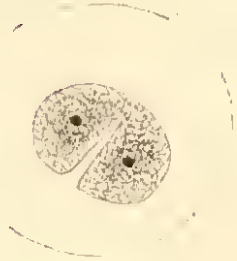

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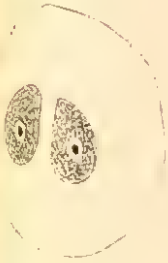

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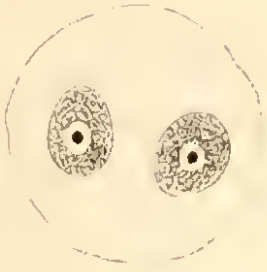

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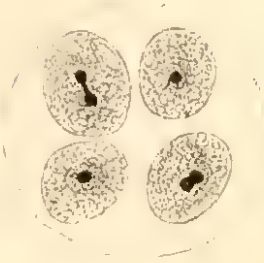

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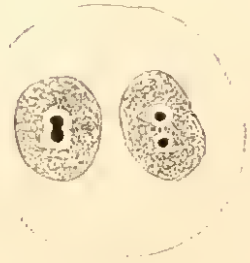

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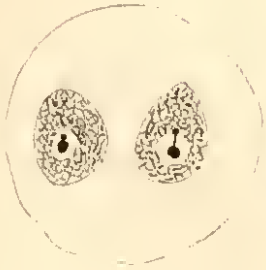

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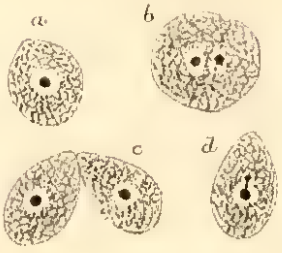

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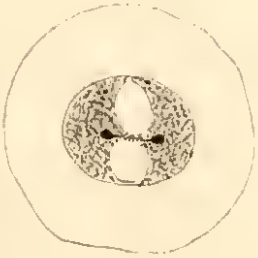

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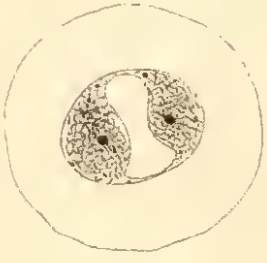

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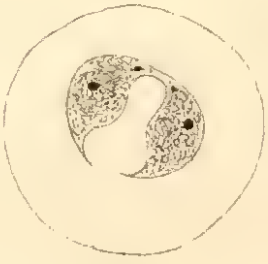

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PLATE IX

Figs. 49-78.—Heidenhain-Breinl's stain.

Figs. 49-50.—End stages of pear-shaped division.

Fig. 51.—Division of small nuclei before separation of daughter cells.

Fig. 52.—Division of large nuclei before separation.

Fig. 53.—Formation of a second small nucleus (*n*).

Figs. 54-78.—Formation of large biflagellate parasites.

Figs. 54, 55.—Swelling up of nuclei, in intra-cellular parasites.

Fig. 56.—Division of enlarged small nucleus.

Fig. 57.—Extrusion of chromatin from the nucleus.

Figs. 58, 59.—Swelling up of nucleus in three parasites.

Fig. 60.—Transformation of large nucleus.

Fig. 61.—Formation of flagella.

Figs. 62-64.—Stages in the extrusion of chromatin from the nucleus.

Fig. 65.—Formation of second flagellum.

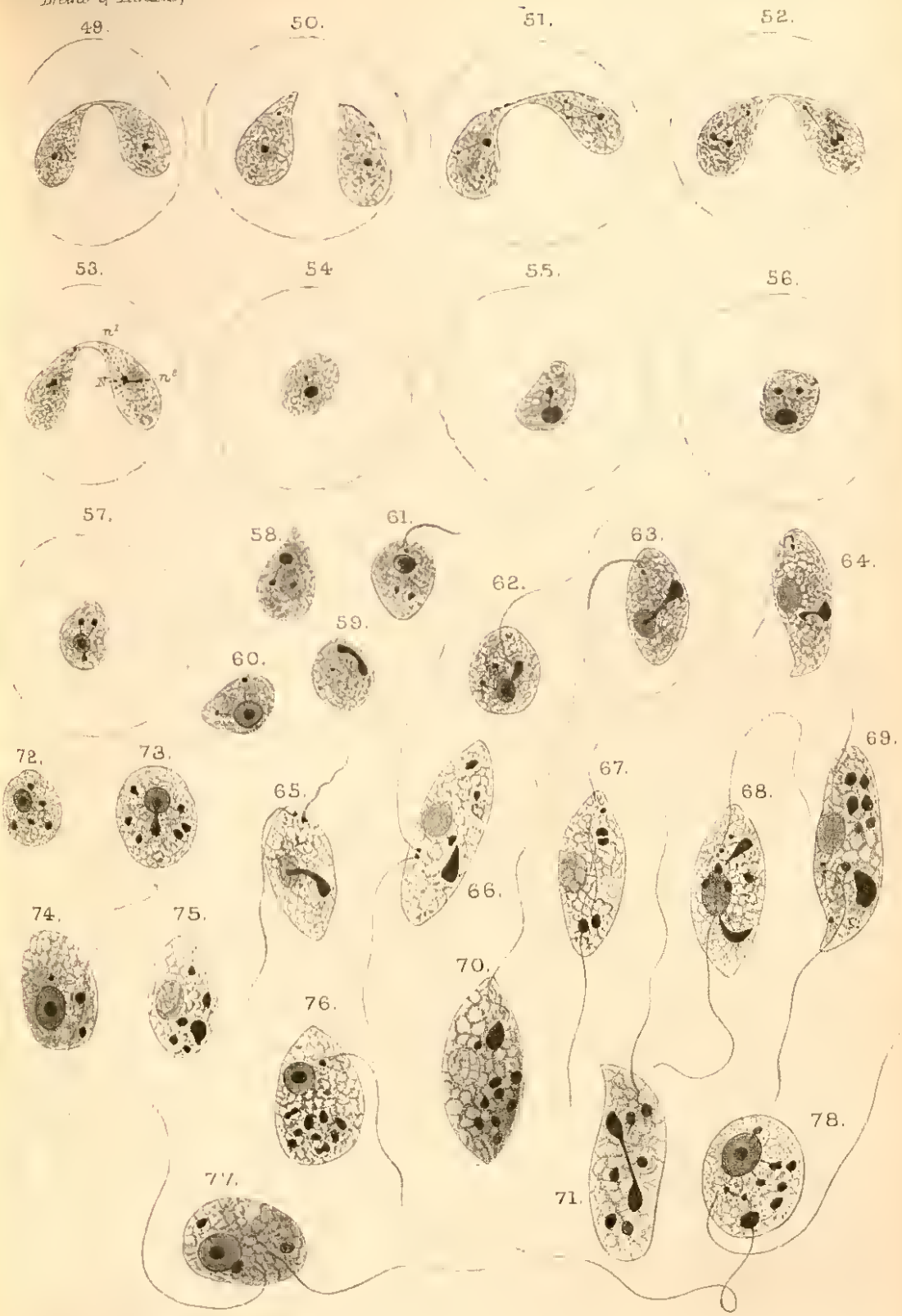
Figs. 66, 67 and 69.—Large elongated biflagellate forms.

Fig. 68.—Extrusion of two masses of chromatin from the nucleus.

Fig. 70.—Large flagellate form after extrusion of chromatin, containing a number of large granules.

Fig. 71.—Division of extruded chromatin and disappearance of the remains of the original nucleus.

Figs. 72-78.—Stages in the development of large biflagellate forms with characteristic nuclei.



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